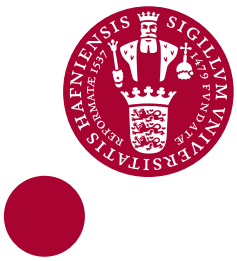

Practical benefits of dormant bud cryopreservation for genetic resource conservation

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talk will outline the extent of current molecular knowledge and give insights into tissue differences in acclimation and evidence for a role for sucrose (in addition to well-known direct protective effects) in regulating cold-responsive gene expression.

THERMAL CHARACTERISTICS OF SOME VITRIFICATION SOLUTIONS

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Different vitrification solutions are used for plant material dehydration and/or cryoprotection by different cryopreservation methods. Differential Scanning Calorimetry (DSC) was used to define thermal characteristics of some vitrification solutions and their components. A first-order phase transitions (melting and crystallization) and a second-order phase transition (glass transition) of sucrose as a basic component of most vitrification solutions was measured at different concentrations. Exothermic events during cooling of sucrose solution were observed at low or medium concentrated solutions up to the eutectic point (63 % w/w sucrose). Two glass transitions at -31°C and -46°C were detected during warming at low sucrose concentrations up to 50 % (w/w). No exothermic event was observed during cooling when the sucrose concentration increased above the eutectic point. Glass transition of highly concentrated sucrose solution (78 % w/w) was -43°C. Glycerol at low concentrations decreased freezing point of solutions more effectively in comparison with sucrose. No exothermic or endothermic events were found in PVS3 solution. No exothermic event was found in the modification of PVS3 solution (40 % w/v glycerol, 40 % w/v sucrose) during cooling but small exothermic and endothermic events were observed during warming in this solution. Lower concentrations of the PVS3 components resulted in exothermic event presence already during cooling. Similar differences in thermal characteristics were found in PVS2 solution and its modifications with lower concentration of basic components.
(Project OC08062 - Thermal analysis - a tool for cryopreservation efficiency improvement)

PRACTICAL BENEFITS OF DORMANT BUD CRYOPRESERVATION FOR GENETIC RESOURCE CONSERVATION

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Many woody plants offer the possibility of direct cryopreservation of dormant, winter buds following natural, or supplemented hardening regimes. Collections working as a genebank e.g University of Copenhagen with 1700 accessions of fruit crops) can optimize the use of human and material resources by devolving collection, cryopreservation and recovery of accessions (by grafting onto an appropriate rootstock) to field staff during the winter season. This reduces significantly pressure on gene bank laboratory staff and the micropropagation facility. The absence of any extended period of *in vitro* culture also limits risks to genetic stability. A further benefit is that recovered whole buds can be grafted directly on to the required rootstocks, whereas recovered in vitro apices, for example, would have to be raised to independent plants of a significant size before grafting, otherwise micrografting

would have to be employed. The technical requirement is to ensure survival of both bud and cambial tissue, to provide the graft union, in a single protocol and, where this is not currently successful, viable buds can be excised from the explants and recovered in a single cycle of *in vitro* whole bud culture. Conventionally cryopreserved, *in vitro* material will be tested for disease status at an appropriate point in the production cycle before distribution and field establishment, and the same can be done for post-recovery, grafted material.

Consequently, this technique can provide significant, and welcome, operational resource benefits to many woody plant conservation programmes, particularly where temperate fruits are concerned. The involved labour force is significantly enhanced, costs of preparation and preservation drastically reduced and the time to field establishment of the required, grafted plant shortened. The usage of the micropropagation facility is also significantly reduced, reducing cost to the field facility and liberating valuable laboratory time that would otherwise be used for routine, not research-level, procedures. The merits, and disadvantages, of this system will be discussed.

EPIGENETIC CHANGES ASSOCIATED WITH THE CRYOPRESERVATION OF CLONAL CROPS

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Cognate with the implementation of COST Action 871 'CRYOPLANET' is Objective 5: to assure the genetic stability and 'true to typeness' of plants after cryopreservation as described within the fundamental aspects of the WG1 package. As tissue culture continues to play a vital role in the development of cryopreservation techniques, there remain challenges regarding the detection of genomic change. Therefore, it is important to assess whether plant germplasm surviving cryogenic storage is genetically identical to the material prior to cryostorage. Consequentially, there is an increasing requirement to determine whether plants derived from cryopreservation are 'true to type' and to measure the extent of the 'normal phenotype' in cryopreserved plants and estimate the degree of closeness to the 'true' parental genotype. These determinations may be achieved through the application of a range of analytical techniques to examine changes at the phenotypic, histological, cytological, biochemical and molecular levels. Regarding the use of investigative tools, the 'state-of-the-art' analytical technology of the most widely used methods is not without criticism (1). Technical limitations exist based on the 'PCR-type' analysis of the primary DNA sequence as these reflect only a small fraction of the plant genome analysed. Therefore, there is a requirement for the presently available techniques to become more genomically widespread in their analysis, also particular PCR-based approaches are unlikely to reveal other changes mediated by epigenetic mechanisms potentially creating a 'gap' in fundamental knowledge. This presentation will consider three clonally propagated crops, blackcurrant (*Ribes*), garlic (*Allium sativum*) and potato (*Solanum tuberosum* L.) and examine the application of different cryopreservation protocols in relation to changes in their epigenetic status. The role of DNA methylation changes during the acclimation and cryopreservation of *Ribes* shoot meristems using genotypes with differential recoveries will also be presented (2). These studies showed DNA methylation was induced in tolerant genotypes and demethylation was evident in the